OXYGEN AS LIMITING FACTOR OF THE PROTOPLASMIC STREAMING IN AVENA COLEOPTILES OF DIFFERENT AGES

by

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In the course of the investigations of the influence of temperature on vital processes it has been observed that at low temperatures the Q_{10} is about constant, while at higher temperatures it gradually decreases. Several explanations have been offered to explain this decrease. During the last few years two schools draw the attention, the one of Crozier (1924 and following years), the other of Belehrádek (1932). Crozier and his collaborators are of opinion that a curve with decreasing Q_{10} should be regarded as a compound of some parts, each of which should agree fully with the formula of van 't Hoff—Arrhenius, while the "temperature-characteristic" (μ), should have a different value for each part. They demonstrated the possibility of this supposition in a large number of cases, representing usually very complicated processes. Belehrádek, however, is of opinion that in all cases where an influence of temperature is studied, a change in viscosity of the protoplasm is the fundamental process. He assumes that the decrease of the Q_{10} with a rise in temperature will take place gradually only. Wassink (1934) has given a valuable contribution to the solution of this problem, after van den Honert (1930) had pointed out that the temperature-relation can be used for the analysis of limiting processes. Wassink analysed the temperature-curve for the respiration of *Phycomyces*, where de Boer (1928) had found a rectilinear relation between temperature and respiration. In this case the Q_{10} already decreases rather rapidly at lower temperatures. Wassink was able to indicate that this curve is the resultant of two van 't Hoff-curves, one with a high and the other with a low Q_5 (Q_5 = 1.60 and 1.10 resp.), older parts of the mycelium producing the former curve, young parts the latter. He could demonstrate that in young cultures the food

supply (i.c. glucose) limits the respiration-velocity, an additional limitation by the oxygen supply also being possible; in the older cultures, however, respiration s. str. was limiting.

When studying the protoplasmic streaming in the coleoptile of *Avena* (1934) I observed that the velocity of the streaming movement did not increase above a certain temperature in young objects, while this clearly could be noticed in old plants. A conclusion to parallelity between these observations with those of Wassink was obvious. This was investigated by studying the influence of the temperature on the rate of protoplasmic streaming in coleoptiles of different ages.

Method.

The coleoptiles are cut lengthwise and the parts are placed on their cut surfaces on an object glass. They were kept in position by a slight pressure on the cover glass. The object glass with the preparates was placed in a microthermostat of Hille Ris Lambers (1926). The streaming in the epidermis was studied. In a Petri-dish covered with moist filter paper and kept at 23° such objects show protoplasmic movement during another 7 days.

The age of the material is indicated in hours, calculated from the beginning of soaking of the grains. Plants which were 72 hrs. old proved to be the youngest that showed measurable streaming; the oldest coleoptiles in which the movement could be measured were about 500 hrs. old. After 600 hrs. streaming still does proceed but it has become very erratic. Still older coleoptiles start drying out.

Four experiments were made at every age, the means of the determinations are produced. As the temperatures at which the streaming velocity was measured were not exactly the same in every experiment, a comparison of the experimental results was realised by calculating the Q_5 for the 5° intervals. The mean velocity of protoplasmic streaming at 13°, calculated from all observations (except those on 72 hrs. old material), was 6.2 μ/sec. This value was employed in the construction of the curves of fig. A, and the velocity at other temperatures was calculated with aid of the mean of the four Q_5 values which were determined for every temperature interval.

Figure A shows that the whole temperature curve of 72 hrs. old plants has a low Q_5, while plants 384 and 450 hrs. old produce a van 't Hoff curve which has a high Q_5 value over its whole range.

Between 3° and 33° the temperature curve of old coleoptiles agrees fully with van 't Hoff's formula, which opposes the obser-

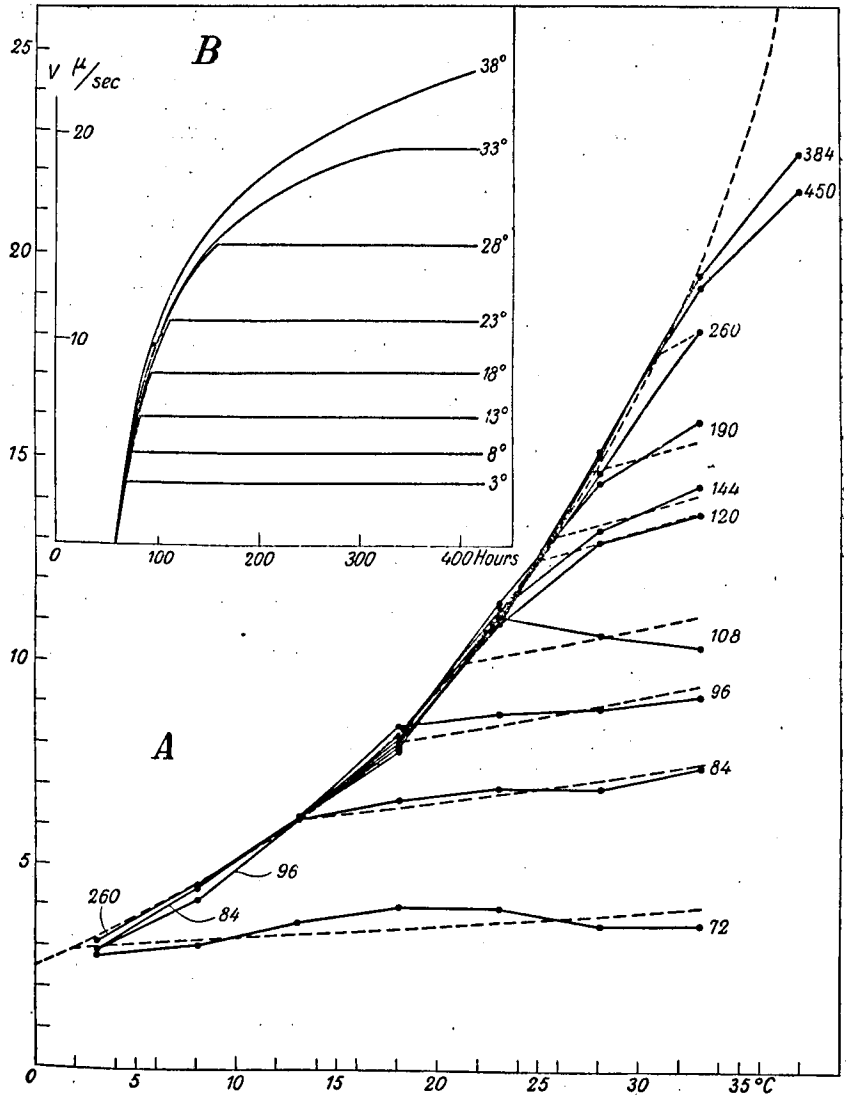

Fig. A. Influence of the temperature on the velocity of protoplasmic streaming. The figures indicate the age of the material in hours. The dotted lines are van 't Hoff curves with $Q_{23}^{28} = 1.33$ and 1.05 respectively.

Fig. B. Relation between the streaming velocity and age of the material at different temperatures, in normal tap water.

vations of other investigators, in particular those of Hille Ris Lambers on *Characeae*. At intermediate ages the produced curve can be explained reasonably to be composed from the two van 't Hoff curves. Thus: *in plants from 84 to 260 hrs. old, at a given moment a certain process, hardly influenced by the temperature, becomes limiting.* The velocity of this process increases with the age of the material (cf. fig. B), consequently the limiting influence becomes active only at increasing temperatures. In some cases intermediate stages occur (120, 144, 260 hrs.). Very likely the temperature at which the limiting factor becomes active in these cases lies between two points of observation. A second intermediate stage, however, would invalidate the supposition that an absolute limiting process was being investigated.

The observations at 38° are of relatively little value in this connection, as at 39°—40° already the protoplasma stops moving (Sachs' "vorübergehende Wärmestarre"). In young plants protoplasmic streaming stops already at lower temperatures; the exact temperature at which this occurs appears to be dependant on still unknown factors, since it happened at different temperatures on different days.

It had to be determined now which process was limiting the protoplasmic streaming in young plants. The following observation gave an indication: a coleoptile, 96 hrs. old, was placed under a cover glass in a drop of water, at a temperature of 23°. The protoplasmic movement, at first clearly visible, came to a standstill after about one minute, but began again when fresh water was sucked past the object. This could be repeated several times. A coleoptile, 260 hrs. old, under the same conditions showed a pronounced streaming during several hours. From the figure A one may derive that in the young coleoptile at 23° the process with low Q_5 value was limiting; in the old one this was no longer so. It was possible that in the first instance the oxygen supply determined the rate of streaming. To investigate this, a circulation apparatus containing the coleoptiles was placed in the microthermostat. The water of the thermostat could not come into contact with the objects, but different liquids could be forced past them.

Water charged with nitrogen and with oxygen respectively were employed. To obtain this, an Erlenmeyer flask of 1 l. was filled with tap water. The water was made to boil to remove the dissolved air in it. During subsequent cooling nitrogen gas from a cylinder was passed through, while the flask was tightly closed after cooling. Commercial nitrogen as employed contains about

4% oxygen, about 7% of the dissolved gas will have been oxygen as against 34% in water saturated with air. In a similar way 1 l. water was saturated with oxygen. By nitrogen or oxygen pressure respectively the liquid was forced through the circulation apparatus.

The results have been tabulated.

Influence of different gas pressures on the rate of protoplasmic streaming.

Plant 260 hrs. old	Streaming velocity		Plant 96 hrs. old	Streaming velocity	
Water saturated with	21°	26°	Water saturated with	21°	26°
air	10.2	13.4	air	10.1	10.0
nitrogen	9.0	9.1	oxygen	9.9	13.2

It appears that in old plants a reduction of the oxygen pressure may cause a limitation of the streaming velocity, while in young coleoptiles an increased oxygen pressure reduces the limiting process. The figures of the table show that the limiting effect has been eliminated altogether (Q_{21}^{26} in oxygen being 1.33).

The oxygen concentration in the medium limits the rate of protoplasmic streaming in young plants. Very likely the oxygen diffusion is here the limiting process. This is contradictory to the results of former investigators, who found, that protoplasmic movement still takes place at very low oxygen pressures (cf. Ewart, 1903), but as they chiefly worked with green plants, oxygen produced by assimilation may have influenced their results.

From fig. B may be deduced that diffusion of oxygen becomes easier with increasing age of the objects, or that less oxygen is required in such material to maintain a certain rate of protoplasmic movement.

It is very likely that in coleoptiles of *Avena* protoplasmic streaming is closely related to respiration. The next indicated step would be to investigate whether respiration itself in young coleoptiles is limited by the oxygen supply but, at present, time fails me. Attention must be drawn to the fact that the Q_{10} of respiration usually is about 2.5, while here for the Q_{10} of protoplasmic movement only 1.8 is found.

The difference in behaviour between young and old coleoptiles as described for *Avena* was also noticed in *Andropogon sorghum* Brot, as indicated in the following figures: plant 96 hours old Q_5: $\frac{13^0}{18^0}$=1.40, $\frac{18^0}{23^0}$=1.77, $\frac{23^0}{28^0}$=0.99, $\frac{28^0}{33^0}$=0.94, plant 260 hours old Q_5: $\frac{13^0}{18^0}$=1.68, $\frac{18^0}{23^0}$=1.75, $\frac{23^0}{28^0}$=1.32, $\frac{28^0}{33^0}$=1.38, $\frac{33^0}{38^0}$=1.20. Here the Q_5 values at lower temperatures, however, are higher than in *Avena*.

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